Intragenic amplification of PAX5: a novel subgroup in B-cell precursor acute lymphoblastic leukemia?

Claire Schwab,1,* Karin Nebral,2,* Lucy Chilton,1 Cristina Leschi,3,4 Esmé Waanders,5 Judith M. Boer,6 Markéta Žaliová,7,8 Rosemary Sutton,9 Inggerd Ivanov Överholm,10 Kentaro Ohki,11 Yuka Yamashita,12 Stefanie Groeneweld-Krentz,13 Eva Frohková,8 Marleen Bakus,14 Joelle Tchinda,15 Thayana da Conceição Barbosa,16 Grazi Fazio,17 Wojciech Mylnarski,18 Agata Pastorczak,18 Giovanni Cazzaniga,17 Maria S. Pombo-de-Oliveira,16 Jan Trka,8 Renate Kirschner-Schwabe,13,19 Toshihiko Imamura,20 Gisela Barbany,10 Martin Stanulla,21 Andishe Attarbaschi,22 Renate Panzer-Grümayer,2 Roland P. Kuiper,5 Monique L. den Boer,6,23 Hélène Cavé,3,4 Anthony V. Moorman,1 Christine J. Harrison,1,† and Sabine Strehl,2,† on behalf of the International Berlin-Frankfurt-Münster (I-BFM) Study Group

1Leukaemia Research Cytogenetics Group, Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom; 2Children’s Cancer Research Institute, St Anna Kinderkrebsforschung, Vienna, Austria; 3INSERM Unité Mixte de Recherché 1131, Institut Universitaire d’Hematologie, Université Paris Diderot, Paris Sorbonne Cité, Paris, France; 4Département de Génétique, Hôpital Robert Debré, Assistance Publique–Hôpitaux de Paris, Paris, France; 5Department of Human Genetics, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands; 6Department of Pediatric Oncology, Erasmus MC–Sophia Children’s Hospital, Rotterdam, The Netherlands; 7Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel, Germany; 8Childhood Leukaemia Investigation Prague, Department of Pediatric Hematology/Oncology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic; 9Children’s Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, Australia; 10Clinical Genetics Section, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; 11Department of Pediatric Hematology and Oncology, National Center for Child Health and Development, Tokyo, Japan; 12Clinical Research Center, Nagoya Medical Center, National Hospital Organization, Aichi, Japan; 13Division of Oncology and Hematology, Department of Pediatrics, Charité–Universitätsmedizin Berlin, Berlin, Germany; 14Department of Hematology, Universitätsklinik Zürich, Zürich, Switzerland; 15Pediatric Hematology-Oncology Program, Research Center, Instituto Nacional de Cancer, Rio de Janeiro, Brazil; 16Centro Ricerca Tettamanti, Clinica Pediatrica, Università di Milano-Bicocca, Monza, Italy; 17Department of Pediatrics, Oncology, Hematology and Diabetology, Medical University of Lodz, Lodz, Poland; 18German Cancer Consortium and German Cancer Research Center, Heidelberg, Germany; 19Department of Pediatrics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan; 20Department of Pediatric Hematology and Oncology, Medical School Hannover, Hannover, Germany; 21Department of Pediatric Hematology and Oncology, St Anna Children’s Hospital, Medical University of Vienna, Austria; and 22Dutch Childhood Oncology Group, The Hague, The Netherlands

Key Points

- Intragenic PAX5 amplification defines a novel, relapse-prone subtype of B-cell precursor acute lymphoblastic leukemia with a poor outcome.

Introduction

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common childhood malignancy, characterized by a wide spectrum of genetic abnormalities, which are used in risk stratification for treatment.1 PAX5 encodes a transcription factor, which plays a key role in B-cell commitment and maintenance2 and is frequently (20% to 35%) deleted or mutated in BCP-ALL.3-5 Germline PAX5 mutations also occur in familial ALL.6,7 Furthermore, chromosomal rearrangements involving PAX5 result in the expression of potentially oncogenic PAX5 fusion genes.8-12 Here we present a subset of patients with BCP-ALL lacking the major cytogenetic abnormalities (ETV6-RUNX1, BCR-ABL1, and TCF3-PBX1 fusions, high hyperdiploidy, near-haploidy, low hypodiploidy, MLL rearrangements, or intrachromosomal amplification of chromosome 21)1 with intragenic amplifications of PAX5 (PAX5AMP).

Methods

Patients in this study originated from 15 international study groups. All participating centers obtained local ethical committee approval and written informed consent in accordance with the Declaration of Helsinki. Diagnosis of BCP-ALL was confirmed by immunophenotyping, according to standard criteria. Demographic and clinical details are summarized in supplemental Table 1.

The copy numbers of individual PAX5 exons were determined using the SALSA multiplex ligation-dependent probe amplification (MLPA) kit P335 IKZF1 (MRC Holland, Amsterdam, The Netherlands), as previously described (supplemental Methods).13-15 Thirteen PAX5AMP samples were processed on
Survival analysis considered event-free survival, defined as time to relapse, and overall survival, defined as time to death, both censored at last contact. Very early relapse was defined as within 18 months of diagnosis, early relapse as >18 months and ≤6 months after the end of treatment, with late relapse defined as occurring >6 months posttreatment. Survival rates were calculated using the Kaplan-Meier method and compared using univariate Cox regression models. All analyses were performed using Intercooled Stata 14.0 (Stata, College Station, TX).

**Results and discussion**

PAX5<sup>AMP</sup> was identified in 79 patients with BCP-ALL, at diagnosis in 77 cases; only relapse material was available from 2 patients (Figures 1 and 2A). The amplified region encompassed exons 2 and 5.
PAX5 DNA-binding and octapeptide domains (Figure 1). The extent of individually to this study. Apart from 1 patient case with (occurring in 52 of 5535 patients from population-based cohorts located within the PAX5 deletions (n = 8). Because this MLPA approach did not target exon 2 or 5 amplification. In patient 31, the probe ratio values for exons 2 and 5 were just below the cutoff of 2 for ≥4 copies; because the percentage of blast cells was low at 83.5%, this result was interpreted as amplification. In patient 69, MLPA showed that exon 2 had a ratio of 2.42 and exon 5 of 1.69; however on the single-nucleotide polymorphism array, exons 2 to 5 were amplified. (B) Data from 9 matched diagnosis-relapse pairs. *In patient 37, the difference in copy number of the amplified exons between diagnosis and relapse was due to reduced percentage of blasts at relapse. **P2RY8-CRLF2 fusion assessed by MLPA, FISH, and/or reverse-transcriptase polymerase chain reaction. ***Patient 16 presented with partial trisomy of chromosome 5 as a result of an unbalanced translocation involving chromosomes 1 and 5: 47,XY,der(1)(1qter-1p21::5q?34-5qter), der(5)(5pter-5q15::1p21-1pter).

Among the other genes assessed by MLPA, CDKN2A/B loss was the most common abnormality associated with PAX5AMP (82%), higher than in other BCP-ALL subgroups. Gain of EBF1 (26%), deletion of IKZF1 (13%), and deletion of the PAR1 region resulting in P2RY8-CRLF2 fusion (10%) were other common alterations, suggesting a collaborative role in PAX5AMP leukemia development. Consistent with the MLPA data, chromosomal abnormalities involving chromosome arm 9p (26%), trisomy 5 (23%), and monosomy 7 (12%) were observed among patients with successful karyotypes (n = 57; supplemental Table 3). Notably, trisomy 5 is a rare finding in BCP-ALL in the absence of high hyperdiploidy. Our previous study of trisomy 5 as the sole cytogenetic abnormality suggested an association with poor prognosis. The main demographic and clinical features of the 77 patients with PAX5AMP identified at diagnosis were male predominance (66%), age >10 years (25%), white blood cell count (WBC) ≥50 × 10^9 (39%), and National Cancer Institute high-risk status (55%). Minimal residual disease (MRD) data were available for 45 patients. Among ALL2003 fusion, PAX5AMP was mutually exclusive of other major risk-stratifying cytogenetic markers, including IGH, PDGFRB/CSF1R, ABL1, ABL2, JAK2, and ZNF384 rearrangements, among 24 patient cases tested by FISH (data not shown).
patients with evaluable MRD (n = 8), 50% were positive at day 28 (≥0.01%). Among patients treated in ALL-BFM 2000 with MRD data (n = 14), 12 were classified as MRD intermediate risk and 1 each as high and low risk, whereas all European Organisation for Research and Treatment of Cancer patients (n = 10) were intermediate risk, apart from 1 classified as high risk. From these limited data, we cannot assign an association between PAX5\textsuperscript{AMP} and MRD.

Among 74 patients with complete remission data available, 73 achieved complete remission by end of induction; 1 patient died before therapy. Relapse occurred in 40% (29 of 73) of these patients. The site of relapse, known for 22 patients, was isolated bone marrow (n = 16), extramedullary (n = 3), or combined relapse (n = 3). The time to relapse (median, 2.1 years) was known for 25 patients, with a ratio of very early to early to late relapse of 9:10:6, classifying 15 (55%) as high risk according to current criteria.\textsuperscript{20} Among patients experiencing relapse with sufficiently long follow-up, 17 (59%) died (relapse, n = 9; infection in remission, n = 3; unknown, n = 5), and 10 remained alive >3 years postrelapse.

The 5-year EFS and OS rates, evaluable for 74 patients, were 49% (95% confidence interval [CI], 36%-61%) and 67% (95% CI, 54%-77%), respectively. To identify risk factors, we examined the effects of age, WBC, National Cancer Institute status, year of diagnosis, and presence of additional genetic abnormalities, but only WBC was significant. Patients with a WBC >50 × 10\textsuperscript{9}/L had a significantly increased risk of death (hazard ratio, 3.48; 95% CI, 1.46-8.32; P = .005). In context, these low survival rates were generated from patients diagnosed over a 22-year period (1993-2015), treated according to a wide range of trial protocols, highlighting the need for prospective studies.

The clinical, genetic, and outcome profiles of patients with PAX5\textsuperscript{AMP} were distinct from those harboring PAX5 deletions, which occur at different incidences between BCP-ALL subgroups.\textsuperscript{3} Although present at an increased frequency in high-risk ALL, PAX5 deletions are not associated with an inferior outcome.\textsuperscript{21,22} Because the number of patients with BCP-ALL with distinct PAX5 fusions is limited, their prognostic relevance remains to be determined.

In conclusion, we have identified a rare subset of patients with BCP-ALL with PAX5\textsuperscript{AMP}, who share a distinct spectrum of genetic abnormalities, including high frequencies of CDKN2A/B loss and trisomy 5. A majority of these patients lack established cytogenetic abnormalities, suggesting that PAX5\textsuperscript{AMP} may define a distinct subtype of BCP-ALL. Although several patients presented with P2RY8-CRLF2 and 1 with BCR-ABL1, both have been reported as secondary changes occurring alongside primary genetic abnormalities.\textsuperscript{3,23,24} Where matched diagnosis and relapse samples were available, the same amplification was present at both time points, indicating that PAX5\textsuperscript{AMP} may be an important driver of leukemogenesis. Because patients with PAX5\textsuperscript{AMP} showed a high incidence of relapse, we recommend testing for PAX5\textsuperscript{AMP} in future ALL trials to determine its true prognostic impact.

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Authorship

Contribution: C.S., K.N., S.S., and C.J.H. designed the study; C.S., L.C., K.N., S.S., C.J.H., and A.V.M. analyzed and interpreted data; the remaining authors provided genetic and clinical data; and all authors approved the final manuscript.

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The current affiliation for E.W. and R.P.K. is Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands.

A list of the members of the International Berlin-Frankfurt-Munster (I-BFM) Study Group appears on the I-BFM website (https://bfiinternational.wordpress.com/).

ORCID profiles: R.S., 0000-0002-0188-6005; K.O., 0000-0003-2838-4555; E.F., 0000-0002-6900-8145; J. Tchinda, 0000-0002-9450-2006; G.F., 0000-0001-7077-8422; G.C., 0000-0003-2955-4528; J. Trka, 0000-0002-9527-8608; H.C., 0000-0003-2840-1511; S.S., 0000-0002-0179-0628.

Correspondence: Sabine Strehl, Children’s Cancer Research Institute, St Anna Kinderkrebsforschung, Zimmermannplatz 10, 1090 Vienna, Austria; e-mail: sabine.strehl@ccri.at; and Christine J. Harrison, Leukaemia Research Cytogenetics Group, Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle University, Level 6, Herschel Building, Newcastle-upon-Tyne NE1 7RU, United Kingdom; e-mail: christine.harrison@newcastle.ac.uk.

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